

Comparison of Chromatographic Spot Patterns of Some North American *Isoetes* Species

L. S. KOTT and D. M. BRITTON*

Few comparative phytochemical studies have been done among the species of *Isoetes*. A comparison of the phenolic compounds in some species in the orders Isoëtales, Lycopodiales, and Selaginellales (Vorin, 1972) showed only biflavones, whereas other members of the Isoëtales and Lycopodiales had other types of flavones. Vorin (1972) also isolated the flavones apigenin and luteolin from European *I. delilei* Rothm. and *I. durieui* Bory. Shortly thereafter, Vorin, Jay, and Hautville (1975) isolated the unique flavone, isoetin, in these same two species, and this clearly separated the quillworts from the lycopods chemically. In further work on four European species (*I. durieui*, *I. delilei*, *I. lacustris* L. and *I. velata* A. Braun), seven compounds were isolated: apigenin, luteolin, isoetin, selagin, chrysoeriol, tricetin, and orientin (or isoorientin) (Vorin & Jay, 1978). Each species showed a specific grouping of these compounds.

In other chemotaxonomic studies of *Isoetes* species from the southeastern United States, it was discovered that very little taxonomic information could be gleaned from chromatographs (Matthews & Murdy, 1969, Boom, 1979). They considered that there was more variation between populations within a species than between species.

The present phytochemical study was initiated to survey the patterns of the phenolic compounds as part of a larger taxonomic study of the genus *Isoetes* in northeastern North America (Kott, 1980). No attempt was made to isolate or identify the compounds.

MATERIALS AND METHODS

Populations of *Isoetes* species used in the chromatographic survey were determined morphologically (Kott, 1980) and cytologically (Kott & Britton, 1980). The species examined were: *I. macrospora* Dur., *I. tuckermanii* A. Braun, *I. riparia* Engelm., *I. acadiensis* Kott (Kott, 1981), *I. eatonii* Dodge, *I. echinospora* Dur., and one population of *I. engelmannii* A. Braun. *Isoetes hieroglyphica* A. A. Eaton was not analyzed chemically because too few collections were available of this rare species. In all, 35 populations were sampled, and several duplicate chromatographs were prepared for each population. Extracts were prepared by powdering dried leaves from several plants in a population and soaking 0.1 gm of material in 0.1 cc of absolute methanol for 48 hours. Paper chromatographs were prepared by applying 200 µml of each sample to standard Whatman #1 paper using the spot method. The chromatographs were run ascendingly in butanol:acetic acid:water (BAW) (12:3:5) in one direction (30 hours) and in a 2% formic acid solution in the other direction (6 hours). Dried chromatographs were examined in the presence of long wave ultra-violet light and ammonia vapor. Average R_f values were determined for all obvious and constantly recurring spots (major spots) (Table 1).

*Dept. of Botany and Genetics, University of Guelph, Guelph, Ont. N1G 2W1, Canada.

RESULTS AND DISCUSSION

The spot patterns were not strikingly different overall from species to species, but some differences existed in the presence or absence of certain major spots. The chromatographs of the seven *Isoëtes* species examined fell into one of two spot patterns, the *I. echinospora* type and the *I. macrospora* type.

Isoëtes echinospora, *I. eatonii* and *I. riparia* all generally shared a common pattern with a maximum of 8 major spots (Fig. 1a). Although only one population of *I. engelmannii* was sampled, it perhaps should also be included in this group. It differs by the absence of spots 2, 3 and 7 (Table 1). *Isoëtes eatonii* and *I. riparia* have almost identical patterns to *I. echinospora*, except that the two former species have one spot each that may be weak or absent at times (Table 1).

TABLE 1. ANALYSES OF SPOT PATTERNS FOR SEVEN SPECIES OF *Isoëtes*.

		1	2	3	4a	4b	5	6	7	8
color of spot:	UV	DP	P	P	DP	DP	P	P	P	P
	UV + NH ₃	Y	P	P	YG	YG	Y	OY	P	P
Rf : BAW		.84	.83	.85	.49	.47	.34	.25	.55	.49
Rf : 2% Formic acid		.0	.05	.19	.05	.13	.02	.02	.02	.10
<i>I. echinospora</i>		+	+	+	+		+	+	+	+
<i>I. eatonii</i>		+	±	+	+		+	+	+	+
<i>I. engelmannii</i>		+			+			+		+
<i>I. riparia</i>		+	+	+	+		+	+	±	+
<i>I. macrospora</i>		+	±	+		+	+	+		
<i>I. acadiensis</i>		+		+		+	+	+		
<i>I. tuckermanii</i>		+	±	+			+	+		

Color code: DP = dark purple; P = purple; Y = yellow; YG = yellow-green; OY = orange-yellow

The remaining three species, *I. macrospora*, *I. tuckermanii*, and *I. acadiensis*, generally share the other spot pattern. Basically their pattern resembles the first but with fewer major spots (Fig. 1b). In these three species, the purple spots 7 and 8 present in the *I. echinospora* type are absent. *Isoëtes tuckermanii* lacks spot 4b and *I. acadiensis* lacks spot 2, both of which are present in *I. macrospora* (Table 1).

The large yellow-green spot (4a) at Rf .49/.05 in the *I. echinospora* type of pattern may be a different compound from the yellow-green spot (4b) at Rf .47/.13 in the *I. macrospora* type pattern. Until the compound is analyzed chemically, it cannot be determined whether these spots are similar, but it appears that this yellow-green spot is consistently in a slightly different position in each of the two patterns.

In conclusion, it appears that gross chromatography can add some taxonomic information to well established morphological and cytological data. Spore morphology of *I. tuckermanii* and *I. macrospora* share some similarities, and it is not unlikely that they share a common ancestor. This is reflected to some extent in the spot pattern that they share. *Isoëtes tuckermanii* has a spot pattern different from that of *I. riparia*, a species with which it is often confused using spore morphology

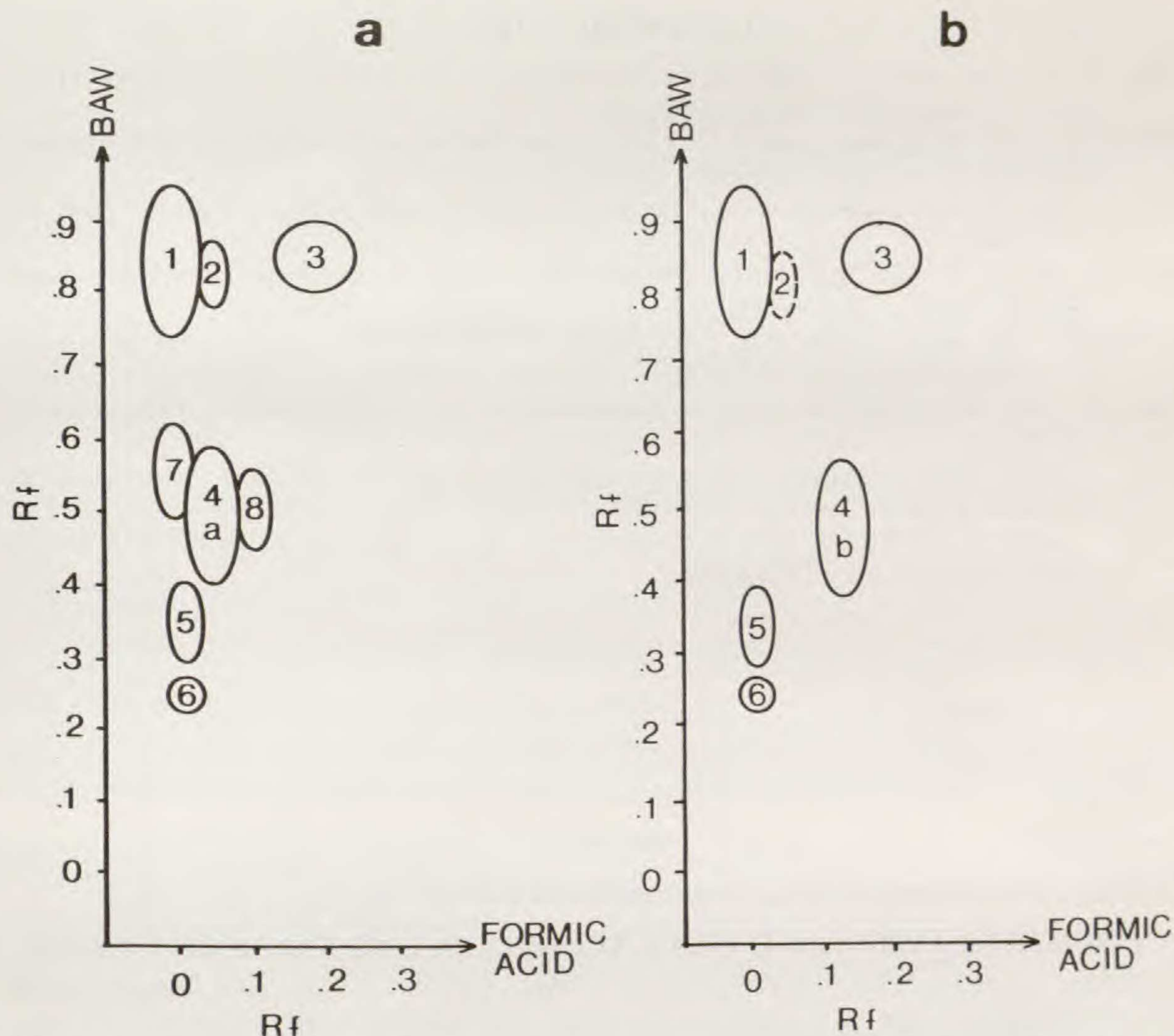


FIG. 1. Comparative spot patterns produced by two dimensional chromatography on *Isoetes* species. a. Composite spot pattern of *I. echinospora* type. b. Composite spot pattern of *I. macrospora* type.

and habitat preference. *Isoetes riparia*, however, has great affinities with *I. echinospora* in spot pattern, and perhaps one of the parents of this tetraploid species is the diploid *I. echinospora*. This is supported by spore morphology of *I. riparia*, which is intermediate between the spiny and the ridged spore type.

The three diploid species *I. eatonii*, *I. echinospora* and *I. engelmannii*, although unique in spore pattern, have similar chromatographic spot patterns. The phytochemical spot pattern appears to yield no further taxonomic information about these species at this level of comparison. Identification of the compounds that produce the spots may lead to a better understanding of the relationships among the groups, since the unique spore morphology of each of the three diploid species indicates that they are not closely related.

We wish to thank Natural Science and Engineering Research Council for financial support for this project.

LITERATURE CITED

- BOOM, B. M. 1979. Systematic studies of the genus *Isoetes* in the southeastern United States. M.Sc. Thesis, University of Tennessee, Knoxville.
- KOTT, L. S. 1980. The taxonomy and biology of the genus *Isoetes* L. in northeastern North America. Ph.D. Thesis. University of Guelph, Guelph, Ontario.
- . 1981. *Isoetes acadiensis*, a new species from eastern North America. *Canad. J. Bot.* 59: 2592–2594.
- , and D. M. BRITTON. 1980. Chromosome numbers for *Isoetes* in northeastern North America. *Canad. J. Bot.* 58:980–984.
- MATTHEWS, J. F. and W. H. MURDY. 1969. A study of *Isoetes* common to the granite outcrops of the southeastern piedmont, United States. *Bot. Gaz.* 130:53–61.
- VORIN, B. 1972. Distribution des composés polyphénoliques chez les Lycopodiées. *Phytochemistry* 11:257–262.
- , and M. JAY. 1978. Etude chimiosystématique des Lycopodiales, Isoëtales, Selaginellales et Psilotales. *Biochem. Syst. Ecol.* 6:99–102.
- , and M. HAUTVILLE. 1975. Isoétine, nouvelle flavone isolée de *Isoetes delilei* et *Isoetes duriei*. *Phytochemistry* 14:247–259.

REVIEW

“**LOUISIANA FERNS AND FERN ALLIES**,” by J. W. Thieret. vi + 123 pp., 75 plates. Lafayette Nat. Hist. Museum. 1980. \$17.95.—This is a semitechnical floristic treatment, and so is useful to botanists and is at the same time understandable to non-specialists. Each species native to or naturalized in Louisiana is treated. The scientific name, sometimes one or more common synonyms, the vernacular name, a very brief description that is supplemented by a plate of several figures, notes, and a general statement of range are given. The keys to the species of each genus are ample and usable. The introductory portion of the book will acquaint non-specialists with some of the intricacies of pteridology. Unfortunately, there is no conventional key to the families or genera. Instead, there is a “guide” consisting of a series of sequential, non-contrasting statements each leading to one or more genera. By reading through the statements, according to the author, “Eventually you will come upon a statement descriptive of the fern or fern ally you wish to identify.” This procedure is roughly twice as cumbersome as a conventional key. The plates, which are reproduced from other publications with permission, are diagnostic, but any grace or delicacy they had as originals has been lost in reproduction. A dot map by parishes (the equivalent of counties) is included on each plate. The plates are grouped near the center of the book and are unpagged, but are numbered. The work concludes with a checklist of Louisiana pteridophytes, a glossary, literature cited, and an index. The book is paper-backed and perfect bound, and so will not withstand extensive rough handling. Presumably the book can be obtained only from the publisher, in Lafayette, Louisiana 70501.—D.B.L.